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Preventing Ototoxic Synergy Of Prior Noise Trauma During Aminoglycoside Therapy

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14. ABSTRACT

Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status.

15. SUBJECT TERMS

Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, ototoxicity, auditory function, hearing loss

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INTRODUCTION

Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status. The combination of both prior noise trauma and aminoglycoside treatment can degrade auditory function greater than simple summation of the two insults. We have found that prior sound exposure enhances cochlear uptake of aminoglycosides, providing a mechanistic basis for the observed ototoxic synergy due to noise trauma and subsequent aminoglycoside treatment.

In the mammalian inner ear – the cochlea, the auditory sensory cells, particularly outer hair cells (OHCs), are more susceptible to aminoglycoside-induced cytotoxicity than other cochlear cells, particularly at the base of the cochlea most sensitive to higher frequency sound. Once these OHCs are lost, these sensory cells cannot be endogenously regenerated, leading to life-long hearing loss and deafness. Thus, extensive efforts are underway to ameliorate and prevent aminoglycoside-induced hair cell death. Under normal physiological condition, aminoglycosides can rapidly cross the blood-labyrinth barrier (BLB) into the cochlear tissues and fluids and enter OHCs through a number of conduits. The best-characterized conduit is permeation through the mechanoelectrical transduction (MET) channel. The MET channel is mechanically-gated by the extracellular, heterodimeric tip links between two stereocilia. Other mechanisms by which aminoglycosides can enter hair cells include endocytosis, and/or other aminoglycoside cation channels (*e.g.* TRP channels) expressed by hair cells besides the MET channel, such as TRPV4 on the apical membranes, or TRPA1 on the basolateral membranes, of OHCs.

The ultimate goal of this research is to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, including combatants and associated casualties to pre-injury effectiveness. In this project, we hypothesize that prior noise trauma induces synergistic ototoxicity with systemically-administered aminoglycosides by potentiating cochlear uptake of the drug. We also hypothesize that specific aminoglycoside-permeant cation channels directly facilitate noise trauma-enhanced uptake of aminoglycosides in the cochlea.

KEYWORDS

Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, ototoxicity, auditory function, hearing loss

ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1: Determine the acoustic parameters that induce noise-enhanced aminoglycoside uptake in auditory sensory hair cells.

Aim 1a: Use whole mount preparations of cochlear sensory epithelia to determine the minimum and optimal acoustic paradigms that enhance hair cell uptake of fluorescently-conjugated gentamicin (GTTR) in mice.

Aim 1b: Use whole mount preparations of cochlear sensory epithelia to determine the optimal acoustic paradigms that enhance hair cell uptake of GTTR in guinea pigs.

This is completed by the end of Year one.

Aim 2: Determine if prior noise trauma modifies intra-cochlear trafficking of aminoglycosides.

Aim 2a: Use cochlear perfusion techniques to determine the contribution of endolymph or perilymph trafficking of aminoglycosides to hair cells with prior noise exposure. GTTR will be administrated either systemically or by scala tympani infusion to the animal.

This is initiated by the end of Year one.

Aim 3: Determine if aminoglycoside-permeant channels on the hair cell apical membrane contribute to aminoglycoside uptake by cochlear hair cells.

Aim 4: Determine if TRP channels on the basolateral membrane of cochlear hair cells also contribute to aminoglycoside uptake.

What was accomplished under these goals?

1) Major activities

a) Conducted experiments to determine the optimal acoustic paradigms that enhance fluorescently-conjugated gentamicin uptake in cochlear sensory epithelia of C57Bl/6 mice.

b) Conducted experiments to determine acoustic paradigms that will potentially convert the sound-enhanced GTTR uptake by tissue that observed in mice to larger rodent model, guinea pigs.

c) Conducted thorough data analysis to document the specie-specific observation of sound-enhanced GTTR uptake by cochlear tissue in guinea pigs.

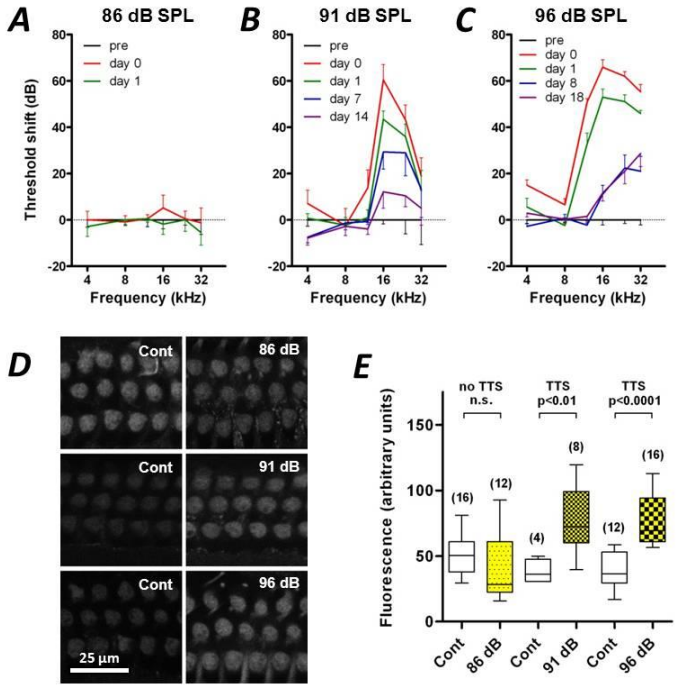


***Figure 1**. Prolonged WBN at 86 dB SPL for 18 hours over three days did not induce TTS (**A**), nor enhance GTTR uptake by OHCs (**D**, top panel comparison; the fluorescence is GTTR). Instead, prolonged WBN at 91 and 96 dB SPL for 18 hours over three days did induce TTS (**B**, **C**) and enhanced GTTR uptake by OHCs (**D**, middle and bottom panel comparisons). Note that experiments at different levels of sound exposure were conducted independently of each other, and with different batches of GTTR, thus GTTR uptake was only comparable within experiments, i.e. horizontally in **D**. Sound-induced TTS was manifested in a broader frequency range from 91 to 96 dB SPL sound treatment (16 to 32 kHz in B, versus 12 to 32 kHz in C). However, sound-enhanced uptake of GTTR was not further elevated at 96 dB SPL (**E**).*

d) Acquired TrpV1 mice (Cat#3770) from Jackson Laboratories. A colony in transition has been established and breeding is ongoing. This colony will ultimately provide littermates of TrpV1 homozygote and heterozygote to determine the contribution of this particular Trp channel to sound-enhanced gentamicin trafficking within the inner ear.

2) Specific objectives

a) To confirm that prolonged prior noise exposure enhances GTTR uptake by sensory cochlear outer hair cells, with our updated software for noise exposure and newly sound calibration.

b) To determine the degree of hearing loss that is induced by each paradigm of noise exposure using separated batches of mice.

c) To acquire Dunkin-Hartley guinea pigs from Charles River Laboratory and establishe hearing sensitivity standard by auditory brainstem response (ABR) measurement in this strain of guinea pigs, using a new ABR acquisition system.

d) To expose guinea pigs to wide band noise (WBN) and reliably produce hearing threshold shift, and document the cellular uptake of GTTR. That is, using our new ABR acquisition system, to investigate the effect of WBN on the hearing sensitivity of guinea pigs, as well as the intra-cochlear trafficking of GTTR.

e) To quantify the GTTR fluorescence signals in the cochlear tissue, including strial tissue (marginal cell layer, intermediate cell layer and basal cell layer) and organ of Corti (outer

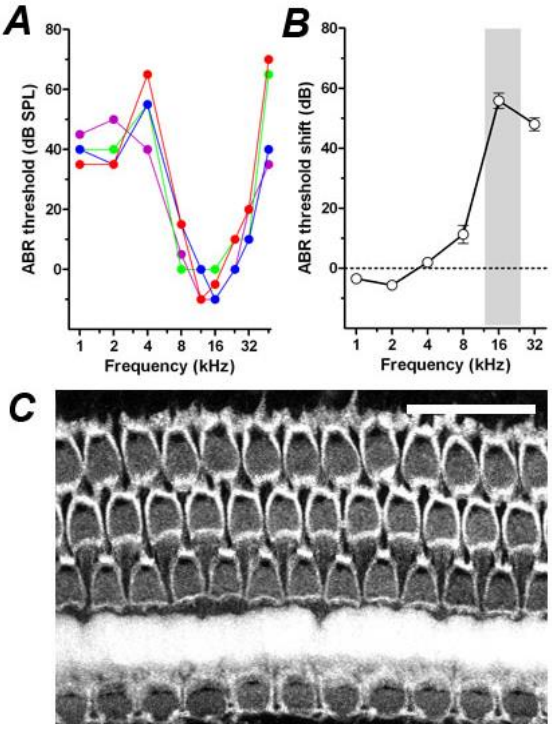


Figure 2*. Sound-induced hearing loss and hair cell survival.* ***A****: Tonal ABR thresholds were evaluated from 4 individual ears of Dunkin-Hartley guinea pigs.* ***B****: Intense OBN sound exposure resulted in elevated ABR threshold shift at high frequencies, including 16 and 32 kHz (n=24). Gray area depicts the OBN. Error bar is SEM. Due to the severity of hearing damage at high frequency region, post-exposure ABR threshold surpassed the limitation of measurement in some tested ears; and the threshold shift was thus underestimated at these frequencies.* ***C****: Sensory hair cells, including outer hair cells (top three rows) and inner hair cells (bottom row) were survived at frequency region of 8 kHz, and below. Scale bar is 25 μm.*

hair cells, inner hair cells and pillar cells). Additionally, to measure the vessel diameter distributions of the strial capillaries in both sound-treated and control animals.

3) Significant results or key outcomes:

a) With new sound calibration, prolonged WBN at 86 dB SPL for 18 hours over three days (interrupted noise condition) did not induce temporary threshold shift (TTS), nor enhanced GTTR uptake by OHCs; while WBN at 91and 96 dB SPL did induced TTS, and enhanced GTTR uptake (Fig. 1).

b) Dunkin-Hartley guinea pigs from Charles River Laboratory exhibited best hearing sensitivity around 12-16 kHz (Fig. 2A). Intense sound exposure, 116 dB SPL octave band noise (OBN), from 12 kHz for 24 hours produced reliable hearing loss at high frequency region, examined 4-weeks later by ABR threshold measurement (Fig. 2B).

c) After WBN exposure at 96 dB SPL of 18 hours (6 hours per day for 3 consecutive days), this strain of guinea pigs exhibit temporary threshold shifts across frequencies (Fig. 3B). However, the most prominent TTS occurred at different time. For instance, at low frequencies such as 2 kHz, the largest TTS occurred 7 days after sound exposure, which was deviant from higher frequencies, such as 12 kHz, where largest TTS occurred 1 day after sound exposure (Fig. 3C).

d) Using WBN exposure at 96 dB SPL of 18 hours (6 hours per day for 3 consecutive days), we have not seen reliable sound-enhanced GTTR uptake by hair cells. Instead, drastic

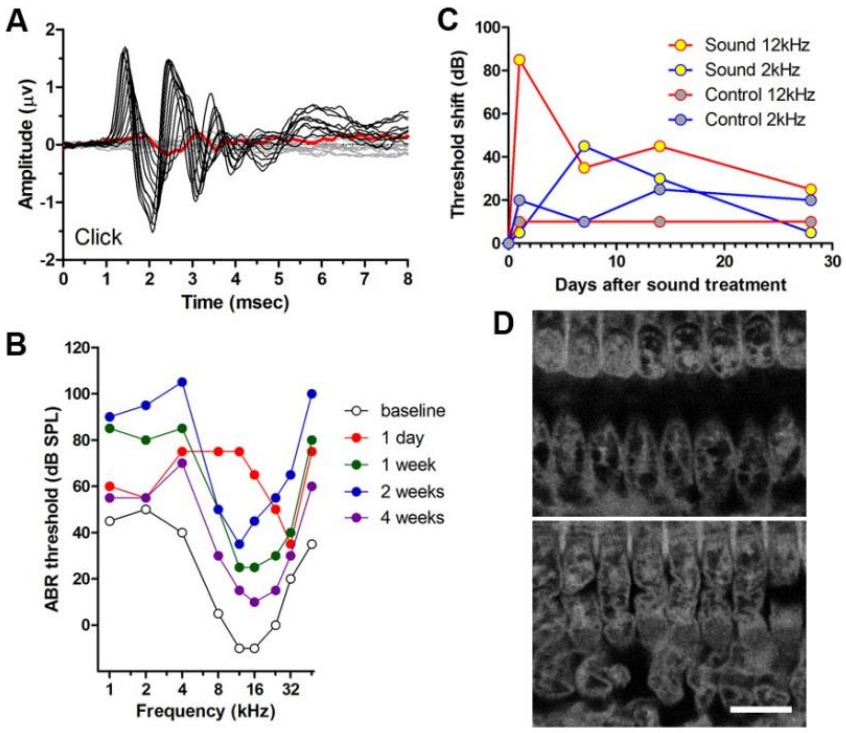


Figure 3*. Sound-induced TTS and GTTR uptake by OHCs.* ***A****: ABR response traces to click at different sound levels, with 5-dB step. Black traces depict supra-threshold responses while gray traces depict under-threshold responses. The thick red trace depicts the threshold which is 55 dB in this example.* ***B****: Tonal ABR thresholds were measured before and after WBN exposure at 96 dB SPL. Different temporal patterns of TTS were observed at lower and higher frequencies, as delineated in* ***C****: largest TTS occurred immediately after sound exposure at 12 kHz, while occurred with delay at 2 kHz. In addition, shifts as large as 20-25 dB occurred in control ears without sound exposure.* ***D****: GTTR uptake by OHCs was observed in 2 kHz region (upper panel) and in 12 kHz region (lower panel). Scale bar is 20 μm.*

changes did occur in the stria vascularis. This includes 1) enhanced GTTR fluorescence signal in basal and intermediate cell layer (Fig. 4C), and 2) vasodilation of strial capillaries (Fig. 4D).

4) Other achievements:

a) We noise exposed mice with single dose sound paradigm at multiple sound levels. The degree of hearing loss was examined, and no overt sound-enhanced GTTR uptake was observed.

b) We examined the organ of Corti from sound-exposed cochleae. Sensory outer hair cells at the frequency region below 12 kHz were survived with intense sound exposure (Fig. 2C), allowing future examination of cellular GTTR uptake. The survival will be guaranteed with the more preferable moderate sound exposure that only induces temporary threshold shift.

c) We observed intensive GTTR uptake by outer hair cells at both lower and higher frequency regions of the cochlea. However, this uptake does not appear sound-enhanced. Examining tissue from more animals will provide statistical power to address the effect of sound exposure.

d) Sound-induced vasodilation was 1) frequency specific, and 2) also observed in mice. In addition, vasodilation appears more evident compared to sound-enhanced uptake of

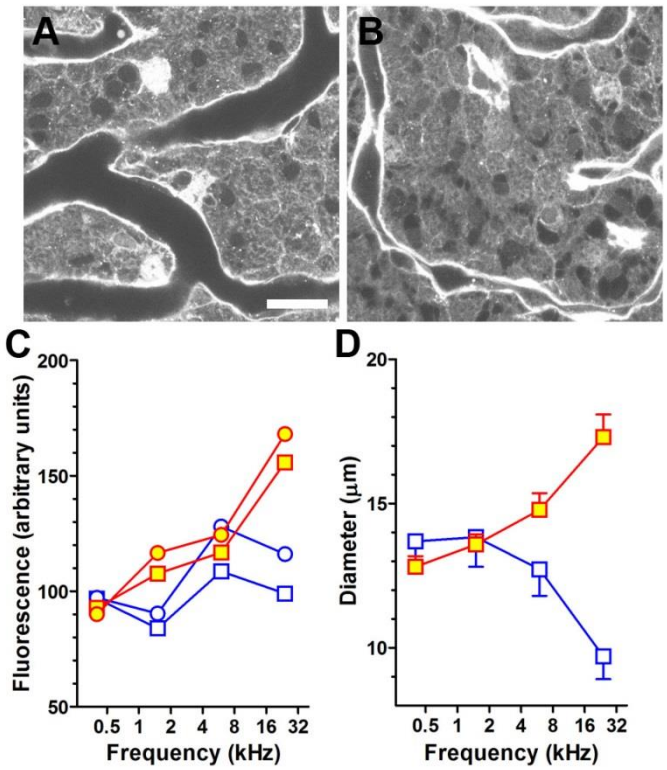


Figure 4*. Sound enhanced GTTR uptake by strial tissue and induced vasodilation within the stria vascularis.* ***A****: GTTR uptake in the strial vascularis from a sound-treated ear at 24 kHz location. The intermediate cell layer is shown here with overt vasodilation. Scale bar is 20 μm.* ***B****: GTTR uptake in the strial vascularis from a control ear at 24 kHz location.* ***C****: Sound enhanced GTTR uptake from the basal cell layer (square) and intermediate cell layer (circle) in the strial vascularis at 24 kHz location, but not at the low frequency locations. Red lines depict the sound-treated ear, while blue lines depict the control ear.* ***D****: Sound induced striking vasodilation of strial capillaries at 24 kHz location. Red line depicts the sound-treated ear, while blue line depicts the control ear. Error bars are 95% confidence interval.*

GTTR in the stria vascularis, thus likely a better morphological marker (or a prerequisite) on the effect of sound potentiated gentamicin ototoxicity.

What opportunities for training and professional development has the project provided?

This research project provided opportunities for college students to get exposure to biomedical research. For instance, Stanley Feng, a sophomore from Oregon State University, involved in this project through OHSU research volunteer mechanism. He participated in the data analysis phase and performed image quantification during Year one. This experience will certainly provide positive impact on his future career decisions with a potential biomedical profession.

How were the results disseminated to communities of interest?

Part of the content in this report has been published at the 2014 Midwinter meeting of Association for Research in Otolaryngology, San Diego, as attached in the Appendix. The same content is also included in a manuscript in preparation, which will soon be submitted to a special issue of Frontier in Neuroscience focusing on cochlear mechanisms. The discovery of sound-induced vasodilation will be published at the 2015 Midwinter meeting of Association for Research in Otolaryngology, Baltimore.

What do you plan to do during the next reporting period to accomplish the goals?

For the upcoming quarter (Year two, Quarter one), we will continue to collect data in guinea pigs, to increase statistic power and assess the statistical significance of sound-enhanced GTTR uptake in the stria vascularis and in the organ of Corti.

For the upcoming year (Year two), we will perform cochlear perfusion experiment to determine if prior noise trauma modifies intra-cochlear trafficking of aminoglycosides, in either guinea pigs or in mice. Additionally, we will use mouse models with MET apparatus defects to conduct experiments to determine if aminoglycoside-permeant channels on the hair cell apical membrane contribute to aminoglycoside uptake by cochlear hair cells.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

During Year one research, we discovered overt vasodilation within a stria vascularis due to acoustic overstimulation. This event appeared more obvious compared to sound-enhanced gentamicin uptake either in the strial tissue or by the hair cells. Thus, vasodilation may serve as a better morphological marker for the elevated risk of sound-potentiated aminoglycoside-induced ototoxicity. In addition, the drastic vasodilation possibly precludes the excessive aminoglycosides gaining access to the vulnerable sensory hair cells within the inner ear, providing potential pharmacological strategies to combat sound-enhance aminoglycoside ototoxicity.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Aminoglycoside antibiotics, like gentamicin and tobramycin, are clinically-essential antibiotics for treating life-threatening Gram-negative bacterial infections. They are being pervasively used, seeing wide application in outpost clinics to national army/veterans hospital. In civilian

population, they are particularly used in premature babies, and for patients with cystic fibrosis, or Gram positive infections like tuberculosis and protozoal infections. Despite their wide use, broad-spectrum, bactericidal efficacy and low cost, clinical dosing with aminoglycosides is limited by the risk of acute nephrotoxicity and life-long ototoxicity, with significant ramifications for quality of life. This research is to search countermeasures to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, as well as civilians received aminoglycoside therapy with a history of (or likely ongoing) acoustic insult.

CHANGES/PROBLEMS

We originally designed the single dose noise exposure (WBN), with a highest sound level of 124 dB SPL. However, after testing, our newly calibrated system only allows a maximum output of ~117 dB SPL. Thus, the highest sound level of single dose noise exposure is now 112 dB SPL, instead of originally proposed 124 dB SPL.

Using the new acquisition system, we obtained ABR threshold in Dunkin-Hartley guinea pigs. The baseline ABR thresholds were below 0 dB SPL at the most sensitive frequencies, such as 12 and 16 kHz (Fig. 1A). This supra-sensitive hearing suggests a potential calibration error within the system. This may not be a problem because we focus on the sound-induced threshold shift, rather than absolute threshold. We have consulted an in-house engineer and experts in electrophysiology, and look into the matter, and thoroughly inspect the system calibration.

Using the new acquisition system, we obtained TTS after WBN exposure in Dunkin-Hartley guinea pigs. The temporal pattern of TTS appears to be frequency specific as depicted in Fig. 3C, which was not previously observed in mice. To examine the GTTR uptake by OHCs, guinea pigs are sacrificed at 0 or 1 day after sound exposure, when the most prominent TTS is observed at higher frequencies but not lower. This may complicate the statistic comparison between sound-treated and control animals. We are now to examine GTTR uptake at corresponding frequency (cochlear) regions. As potential future experiments, we can alternatively perform systemic GTTR treatment one week after sound exposure, and specifically look into the GTTR uptake at lower frequency region (*e.g.* 2 kHz).

As discussed earlier, strial vasodilation appears to be a better benchmark for sound-enhanced GTTR trafficking within the cochlea. We will consider using this benchmark to assess the degree of sound treatment in upcoming experiments of cochlear perfusion.

PRODUCTS

Conference papers and presentations

David Furness, Peter Steyger, Hongzhe Li (2014), "Temporary Threshold Shift Breaks Tip Links in Hair Cells and Enhances Uptake of Gentamicin", Midwinter Research Meeting in Otolaryngology.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Hongzhe Li, PhD
Project Role: PI
Nearest person month worked: 4.8
Contribution to Project: Dr. Li has performed work in experimental design, tissue harvest and processing, confocal imaging, image acquisition and quantification, data analysis, documents, reports and manuscript preparation.

Name: Peter Steyger, PhD
Project Role: Co-Investigator, Professor
Nearest person month worked: 1.2
Contribution to Project: Dr. Steyger has involved in experimental design, animal protocol compliance and manuscript preparation.

Name: Anastasiya Johnson, MS
Project Role: Research Associate
Nearest person month worked: 6.0
Contribution to Project: Ms. Johnson has performed work in ABR recordings in both mice and guinea pigs, and part of image acquisition.

Name: Allan Kachelmeier, MS
Project Role: Research Assistant
Nearest person month worked: 1.2
Contribution to Project: Mr. Kachelmeier has involved in instrument maintenance and document proofreading.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Nothing to report.

APPENDICES

Poster presentation at 2014 Midwinter meeting of Association for Research in Otolaryngology, San Diego.

Temporary Threshold Shift Breaks Tip Links in Hair Cells and Enhances Uptake of Gentamicin

David Furness, PhD[1], Peter Steyger, PhD[2], Hongzhe Li, PhD[2]
[1]Institute for Science and Technology in Medicine, School of Life Sciences, Keele University, Staffordshire, UK
[2]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR, USA

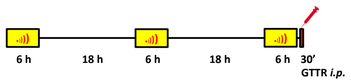
Introduction

Aminoglycoside antibiotics are well-known ototoxic drugs most frequently prescribed for prophylaxis or for treating bacterial sepsis and tuberculosis. Despite over sixty years of clinical use, it remains poorly understood how these drugs traffic from the vasculature into sensory hair cells in various clinical situations. Using cochlear perfusion techniques *in vivo*, we recently demonstrated that systemically-administered aminoglycosides predominantly enter hair cells *via* an endolymph trafficking route [1], prior to exerting their toxicity. Additionally, hair cell uptake of aminoglycosides is increased by prior sound exposure [2], potentially correlating with pre-clinical [3-7] and clinical [8,9] observations on the ototoxic synergy of sound and aminoglycosides.

Exposing to hazardous sound levels, which frequently occur in combat and recreational environments, results in temporary threshold shift (TTS) enhanced gentamicin uptake in cochlear outer hair cells (OHCs) [2]. Thus, sound-enhanced uptake of aminoglycosides by cochlear hair cells is one potential mechanism for the observed ototoxic synergy between sound and aminoglycosides. In this study, we hypothesized that sound-enhanced aminoglycoside uptake occurs *via* OHC mechanoelectrical transducer (MET) channels. To test this hypothesis, we systematically varied the sound level to induce TTS, exposed animals to gentamicin and examined the effect of sound exposure on both drug uptake and tip link survival.

Materials & Methods

To induce TTS, adult C57Bl/6 mice were exposed to wideband noise (WBN; 86-96 dB SPL) for 6 hours/day for 3 days. Mice were then intraperitoneally injected with fluorescently-conjugated gentamicin (GTTR, 2 mg/kg, in PBS, pH = 7.4) to track gentamicin uptake. Thirty minutes later, paraformaldehyde-fixed (4%) cochlear tissues were excised and processed for confocal microscopy and fluorescence quantification. From a different batch of animals, the degree of TTS was assessed by tonal ABR measurements (4, 8, 12, 16, 24 and 32 kHz) before sound exposure, and at multiple time points after sound exposure.



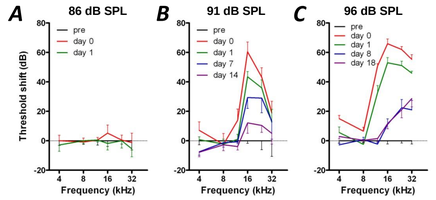
The intensity of GTTR fluorescence was measured in OHCs under identical confocal settings, and processed by Photoshop and ImageJ. Student's T-test was used to determine any significant differences between treatment groups. An independent batch of mice, exposed to 91 dB SPL WBN, were cardiac-perfused with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, and cochlear tissues processed for scanning electron microscopy (SEM). The number of stereociliary tips and visible tip links per hair bundle (10 bundles per OHC row) was counted at a mid-cochlear location corresponding to a frequency range of 12-16 kHz.

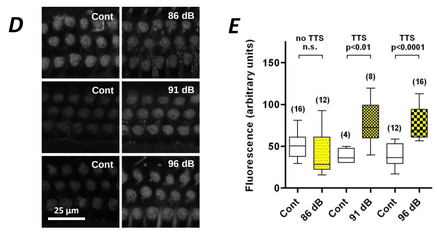
Results

1. TTSs and sound-enhanced GTTR uptake by OHCs

Prolonged WBN at 86 dB SPL for 18 hours over three days did not induce TTS (***A***), nor enhance GTTR uptake by OHCs (***D***, top panel comparison; the fluorescence is GTTR). Instead, prolonged WBN at 91 and 96 dB SPL for 18 hours over three days did induce TTS (***B, C***) and enhanced GTTR uptake by OHCs (***D***, middle and bottom panel comparisons). Note that experiments at different levels of sound exposure were conducted independently of each other, and with different batches of GTTR, thus GTTR uptake was only comparable within experiments, *i.e.* horizontally in ***D***.

Sound-induced TTS was manifested in a broader frequency range from 91 to 96 dB SPL sound treatment (16 to 32 kHz in ***B***, versus 12 to 32 kHz in ***C***). However, sound-enhanced uptake of GTTR was not further elevated at 96 dB SPL (***E***).

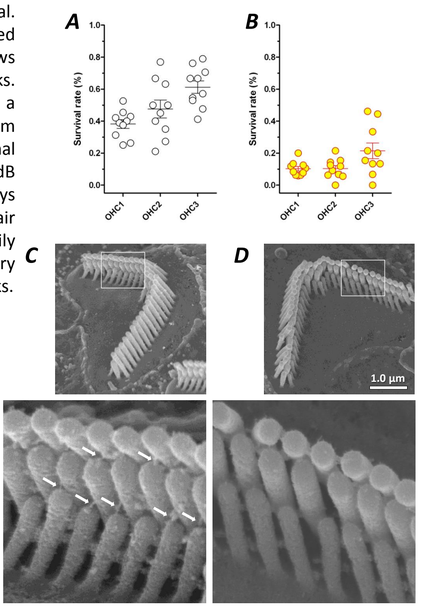




2. Reduced tip link survival after noise exposure

In control animals, we observed variable tip link survival in individual OHC rows from the middle coil (***A***). Each symbol represents tip link survival rate from an individual OHC bundle. Following prolonged WBN at 91 dB SPL for 18 hours, tip link survival rates were reduced (***B***).

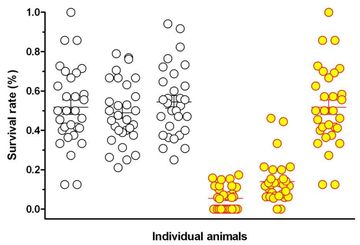
C: The hair bundle of a representative 2nd row OHC from a control animal. Framed area is enlarged below, and white arrows depict identifiable tip links. ***D***: The hair bundle of a representative OHC from the 3rd row of an animal exposed to WBN at 91 dB SPL for 18 over three days showed normal hair bundle structure and easily identifiable stereociliary tips, but *no* visible tip links.



3. Varied tip link survival across animals after noise exposure

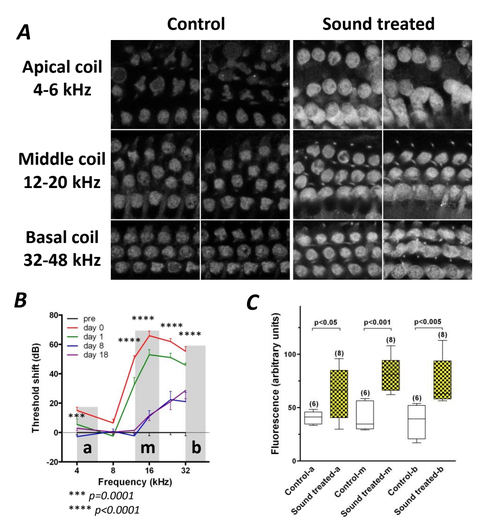
Variable rates of tip link survival were observed in hair bundles from control middle coils (open circles, 3 animals, from all 3 OHC rows of individual animals). After exposure to prolonged WBN at 91 dB SPL for 18 hours over three days, tip link survival was mostly reduced in the middle coil (yellow circles, 3 animals), and the degree of reduction varied among individual animals. We are currently working on the correlations among i) the degree of tip link survival, ii) the severity of TTS and iii) the amount of GTTR uptake.

The MET channel is aminoglycoside-permeant and considered the primary channel utilized by aminoglycosides to load hair cells. A mechanistic question is whether additional non-selective cation channel(s) permeant to aminoglycosides underlie sound-enhanced aminoglycoside uptake. Assuming tip link survival in SEM is indicative of the percentage of tip links present when exposed to GTTR, a reduction in the percent of surviving tip links implies a reduced overall open probability (P_{open}) of MET channels on a hair bundle. However, a reduced P_{open} will hyperpolarize the cell, decreasing auditory sensitivity (*i.e.* TTS), yet increasing the driving force on endolymphatic aminoglycosides to enter OHCs by reducing the drug-MET channel binding constant [11]. The increased drug entry kinetics by residual functioning MET channels may be sufficient to account for sound-enhanced drug uptake *via* aminoglycoside-permeant MET channels alone, without the mechanistic need for additional candidate aminoglycoside-permeant channels.



4. Sound-enhanced GTTR uptake extends beyond TTS region

Micro-dissected cochlear coils were tonotopically mapped *via* an ImageJ plug-in [12]. GTTR pixel intensities in the apical, middle, and basal coils, representing 4-6, 12-20, and 32-48 kHz regions respectively, were obtained (***A***) to compare the level of GTTR uptake with the high-pass shaped TTS curve at various frequency locations. Although the 96 dB SPL WBN induced minimal or no TTS at lower frequencies in the apical coil (***B***), *i.e.* 4-8 kHz, GTTR uptake was significantly greater at this location (***C***). At higher frequency regions, *i.e.* 12-32 kHz, WBN induced threshold shifts up to 70 dB, and more statistically significant increases in GTTR uptake.



Discussion & Conclusions

A wide range of tip link survival rates was observed in the OHCs of control animals, yet the uptake of GTTR by these cells was typically consistent. The relatively homogenous uptake of GTTR and variable presence of tip links involved in gating the aminoglycoside-permeant MET channels in individual hair bundles suggest that tip link survival inversely correlates with a more deeply negative polarization of cell potential. However, it is important to be aware that tip-link survival may be variably affected by the processing of fixed samples for SEM and this could partly account for some of the variability seen.

The sound exposures used here to induce TTS also reduced tip link survival, yet sound-exposed OHCs displayed enhanced GTTR uptake. This suggests that 1) under particular conditions, reduced numbers of MET channels hyperpolarize OHCs enhancing GTTR uptake; or 2) an additional mechanism(s) besides the tip link-gated MET channels facilitates sound-enhanced GTTR uptake. Sound-enhanced GTTR uptake was also observed from a broader, more apical, frequency region than the region with TTS, indicating that further research into the mechanisms of this uptake is warranted.

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